

of *E. coli* ATCC 51183 were inoculated into tryptic soy broth and grown overnight at 37° C. The bacterial culture was diluted to approximately 10⁶ or 10⁵ CFU/ml in Butterfield's buffer (pH 7.2±0.2; monobasic potassium phosphate buffer solution; VWR, West Chester, Pa.) and 100 microliters of the diluted suspension were added directly to individual tubes containing ten ml of deionized water (Milli-Q Biocel System, Millipore, MA) samples to obtain approximately 10⁵ CFU or 10⁴ CFU in ten ml, respectively. Ten mg of autoclaved CM-111 3M™ Cosmetic Microspheres (calcined amorphous spheroidized magnesium silicate powders; 3M Company; St. Paul, Minn.) were added to the tubes containing cells and mixed at room temperature for about 15 min. The particles were allowed to settle and the supernatant was removed. The particles were suspended in 100 µl of Butterfield's buffer and transferred to 1.5 ml microfuge tubes. Four hundred microliters of luciferase/luciferin liquid reagent solution from CLEAN-TRACE surface ATP system was added to the tubes. For the control (unconcentrated) reactions, 100 µl of approximately 10⁶ or 10⁵ CFU/ml cell suspension were added to 1.5 ml microfuge tubes and 400 µl of luciferase/luciferin liquid reagent solution from Clean-Trace surface ATP system was added to the tubes. Immediately after adding the reagent, hydrogel beads (about 11 mg) containing a cell extractant were added to individual tubes and relative light units (RLUs) measurements were recorded at 10 sec intervals in a bench-top luminometer (20/20n single tube luminometer from Turner Biosystems, Sunnyvale, Calif.). Luminescence measurements were obtained from the luminometer using 20/20n SIS software that was provided with the luminometer. The light signal was integrated for 1 second and the results, expressed in RLU, are presented in Table 1.

[0204] The data indicate that, using the protocol described herein, the microparticles were able to concentrate the microbial cells and the cell extractant released from the hydrogel beads was able to extract ATP from *E. coli*. The results further indicate that ATP released from the cells reacted with the ATP-detection reagents, which resulted in measurable bioluminescence.

TABLE 1

Detection of ATP from <i>E. coli</i> cells coupled to a cell concentration agent and exposed to microbial cell extractants released from a hydrogel. Values expressed in the table are relative light units (RLUs).				
Time	Unconcentrated		Concentrated	
(sec)	10 ⁴ Cfu	10 ⁵ Cfu	10 ⁴ Cfu	10 ⁵ Cfu
10	1226	2552	904	1648
20	1239	2648	948	1735
30	1265	2681	1000	1735
40	1272	2820	1067	1786
50	1280	3152	1107	1818
60	1312	3914	1147	1948
70	1352	4960	1178	2139
80	1393	6391	1197	2388
90	1440	8258	1226	2732
100	1538	10230	1250	3188
120	1618	11859	1260	3820
130	1704	12969	1286	4681
140	1838	13527	1297	5842
150	1905	13759	1318	6721
160	2006	13735	1309	6675
170	2088	13762	1314	6513
180	2119	13537	1330	6428
190	2169	13426	1363	6321

TABLE 1-continued

Detection of ATP from <i>E. coli</i> cells coupled to a cell concentration agent and exposed to microbial cell extractants released from a hydrogel. Values expressed in the table are relative light units (RLUs).				
Time	Unconcentrated		Concentrated	
(sec)	10 ⁴ Cfu	10 ⁵ Cfu	10 ⁴ Cfu	10 ⁵ Cfu
200	2140	13353	1375	6220
210	2141	13128	1342	6196
220	2143	13014	1389	6142
230	2155	12903	1381	6076
240	2110	12780	1401	6023

Example 3

Detection of Microbial Cells in a Unitary Sample Preparation and Detection Device Using an ATP Bioluminescence Detection System

[0205] A unitary sample preparation and detection device **200**, as shown in FIG. 2, is used in this Example. The device contains approximately 10 mg of autoclaved CM-111 3M Cosmetic Microspheres in the upper receptacle **220**. Lower receptacle **224** contains a liquid detection reagent **265**, which consists of approximately 0.6 milliliters of the luciferase/luciferin liquid reagent solution from a CLEAN-TRACE surface ATP system. The third receptacle **226** contains two BAR-DAC 205M beads made according to Preparative Example 5 of U.S. Patent Application No. 61/101,546, filed Sep. 30, 2008. Ten milliliters of sterile deionized water is added to the upper receptacle **220** of the unitary devices **200** immediately before use.

[0206] *E. coli* overnight cultures are prepared as described in Example 2. The bacterial culture is diluted to approximately 10⁶ or 10⁵ CFU/ml in Butterfield's buffer. One hundred microliters of the diluted suspension are pipetted directly into upper receptacle **220** of the unitary devices **200** to obtain a suspension of approximately 10⁵ CFU or 10⁴ CFU in ten milliliters, respectively. The cap **278** is used to close the housing **210** and the bacterial suspension is mixed with the microspheres (cell concentration agent **230**) at room temperature and allowed to settle into the passageway **216**. The cap **278** is removed and the plunger **250** is inserted to transfer a portion of the liquid sample containing the settled microspheres and hydrogel beads into the lower receptacle **224**, which contains the ATP detection reagents. The unitary device is immediately inserted into the reading chamber of a luminometer (for example, a NG Luminometer, UNG2) and RLU measurements are recorded at 10 sec interval using the Unplanned Testing mode of the UNG2 luminometer. RLU measurements are collected until the number of RLUs reaches a plateau. The data are downloaded using the software provided with the NG luminometer. The data will indicate that the microbial cells are concentrated by the microspheres, the cell extractant is released by the hydrogel, the cell extractant causes the release of ATP from the cells, and the ATP released from the cells is detected by the ATP detection system.

Example 4

Detection of Microbial Cells in a Unitary Sample Preparation and Detection Device Using an ATP Bioluminescence Detection System

[0207] A unitary sample preparation and detection device **300**, as shown in FIG. 3, is used in this Example. The valve